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The medical work which the Public Health Service was called on to perform for the Bureau of War Risk Insurance and its successor, the United States Veterans' Bureau, is now history. The field organization, including the 14 district supervisors' offices and their sub-agencies, reaching practically into every county of the United States, was turned over in June, 1921. The control of contract hospitals was relinquished also at that time. The Veterans' Bureau, having further perfected its organization, finally felt itself ready to assume the administration of the veterans' hospitals, which were, accordingly, turned over to Director Forbes by Executive order, May 29, 1922. This transfer consisted of 57 hospitals, with 17,500 beds, 13,057 patients (of whom 5,271 were tuberculous), and a personnel of 11,347, including 925 physicians and dentists, 1,425 nurses, 425 reconstruction aides, and 110 dietitians. I take this occasion to pay public tribute, before a body which has ever honored fidelity, to the professional men and women who, having been identified with the organization and operation of these hospitals for many months during very trying periods, were transferred from the Public Health Service to the Veterans' Bureau for continued duty of the same character. Their loyalty has been instant and unvarying, their patience untiring, their zeal unflagging, and their devotion unfailing, in the cause of the disabled veteran and the Government, whose obligations they strive to fulfill.

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### SEPARATION OF TOXIC AND NONTOXIC CELLS FROM CULTURES OF AN ANAEROBE ISOLATED FROM LARVÆ OF THE GREEN FLY.

By IDA A. BENGTSON, Assistant Bacteriologist, United States Public Health Service.

In attempting to obtain an unquestionably pure culture of the spore-forming anaerobe recently isolated from larvæ of a species of the green fly<sup>1</sup> the single cell method of Barber was employed.

The culture used was one developed from a well isolated single colony, fished from a deep liver-agar culture into meat medium, which consisted of one part of chopped meat and two parts of water, the whole adjusted to a reaction of  $p_H$  8.0. This culture was toxic, causing the death of mice in about four hours, in a dose of 0.2 c. c.

A single cell (spore) culture derived from this culture was found to be nontoxic for mice, though the appearance of the growth in meat medium was identical with that of the growth previously obtained. Two other single cells (spores) were isolated; one was found to be toxic and the other nontoxic. These three cultures were designated *a*, *b*, *c*, *a* and *c* being nontoxic and *b* toxic.

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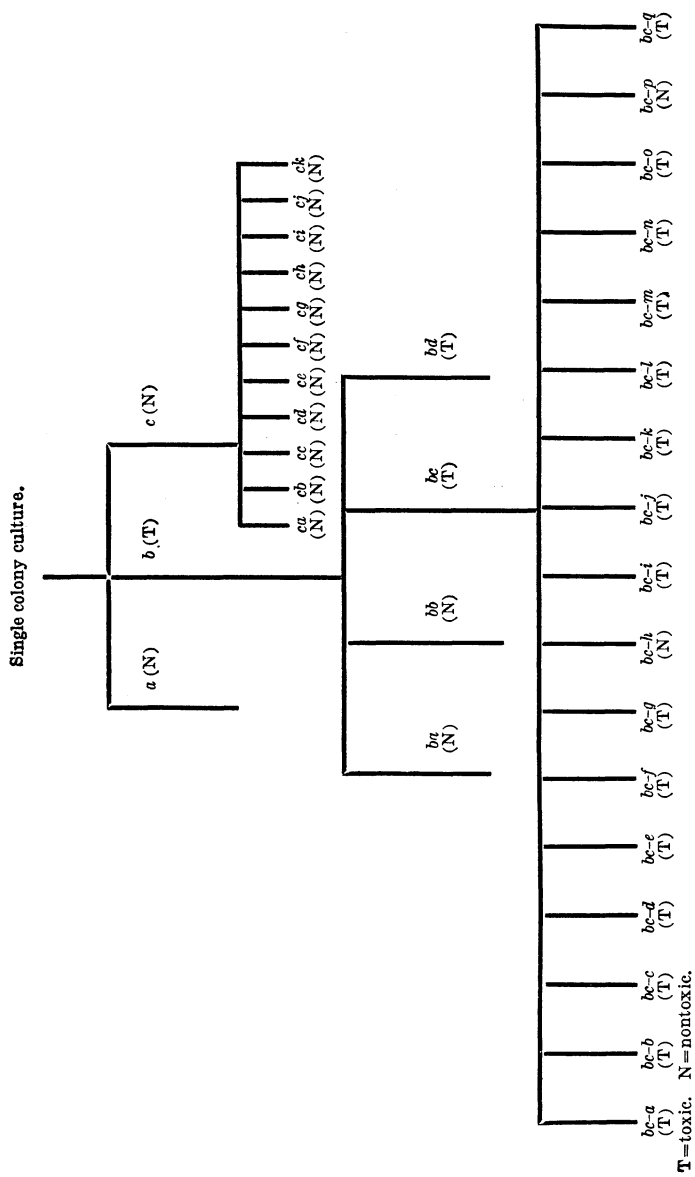
<sup>1</sup>Pub. Health. Rep., 1922, 37, 164-170. Reprint No. 726.

A comparison was made of the morphological and cultural characteristics of the three cultures. The media used included meat medium, litmus milk, liver broth, and ordinary glucose broth in fermentation tubes. Two subcultures of each culture were used. Morphologically, all the cultures were identical, consisting of rods with terminal spores.

All of the cultures showed a similar appearance in the meat medium. The growth in this medium, in contrast to that of single cell cultures of Types A and B of the botulism organism, is distinguished by the absence of any evidence of proteolysis. There is no reduction in the volume of meat, no darkening or blackening of the meat, no comminution of the particles of meat, no darkening of the supernatant broth, and no pronounced odor. A slight acidity was produced in litmus milk, but no coagulation or digestion of casein occurred. All of the cultures failed to grow in glucose broth fermentation tubes. In the liver broth fermentation tubes, culture *a* failed to grow. One of the subcultures of *b* showed a granular growth within 24 hours; but this was precipitated on opening the tube and making a smear, and no further growth occurred. The growths of the second subculture of *b* (toxic) and the two subcultures of *c* (nontoxic) were similar and unusual in that there was no evidence of growth in any of the tubes during the first 10 days. After this time a granular growth appeared. The cultures were left undisturbed and continued to grow, and all had produced gas to the amount of 20 to 25 per cent at the end of two weeks.

The similarity in morphology and in cultural reactions and the unusual behavior in the liver broth indicate the essential cultural identity of the toxic and nontoxic types.

Further single cell isolations were made from cultures *b* (toxic) and *c* (nontoxic). From *b*, two nontoxic cultures (*ba* and *bb*) and two toxic (*bc* and *bd*) were obtained. From *c*, eleven nontoxic cultures have thus far been obtained. From culture *bc*, 17 single cell isolations have been made, 15 of these being toxic and 2 nontoxic. The results are graphically presented in the accompanying diagram.



The two kinds of cultures have remained true to type, with one possible exception. Three months after the beginning of this work all single-cell cultures were retested on mice with the results given below, the subscript figure representing the number of the transplant in meat media. Toxicity tests throughout have been made by inoculation of 0.2 c. c. of the culture intraperitoneally into mice. Characteristic symptoms usually develop within an hour or two and death occurs soon thereafter.

<i>a</i> <sub>9</sub> (N)..... S.	<i>c</i> <sub>8</sub> (N)..... S.
<i>b</i> <sub>9</sub> (T)..... +3 hrs.	
<i>ba</i> <sub>4</sub> (N)..... S.	<i>bc</i> <sub>4</sub> (T)..... +2½ hrs.
<i>bb</i> <sub>3</sub> (N)..... S.	<i>bd</i> <sub>4</sub> (T)..... S. ( <i>bd</i> <sub>1</sub> , <i>bd</i> <sub>2</sub> , <i>bd</i> <sub>3</sub> , all toxic).
<i>bc-a</i> <sub>4</sub> (T)..... +5 hrs.	<i>bc-j</i> <sub>3</sub> (T)..... +3½ hrs.
<i>bc-b</i> <sub>4</sub> (T)..... +2 hrs.	<i>bc-k</i> <sub>3</sub> (T)..... +2 hrs.
<i>bc-c</i> <sub>4</sub> (T)..... +2 hrs.	<i>bc-l</i> <sub>3</sub> (T)..... +7 hrs.
<i>bc-d</i> <sub>4</sub> (T)..... +7 hrs.	<i>bc-m</i> <sub>2</sub> (T)..... +2 hrs.
<i>bc-e</i> <sub>3</sub> (T)..... +3 hrs.	<i>bc-n</i> <sub>2</sub> (T)..... +1 hr.
<i>bc-f</i> <sub>3</sub> (T)..... +4½ hrs.	<i>bc-o</i> <sub>2</sub> (T)..... +2 hrs.
<i>bc-g</i> <sub>3</sub> (T)..... +3½ hrs.	<i>bc-p</i> <sub>2</sub> (N)..... S.
<i>bc-h</i> <sub>3</sub> (N)..... S.	<i>bc-q</i> <sub>2</sub> (T)..... +2½ hrs.
<i>bc-i</i> <sub>3</sub> (T)..... +3½ hrs.	
<i>ca</i> <sub>3</sub> (N)..... S.	<i>cd</i> <sub>3</sub> (N)..... S.
<i>cb</i> <sub>3</sub> (N)..... S.	<i>ce</i> <sub>3</sub> (N)..... S.
<i>cc</i> <sub>3</sub> (N)..... S.	

S=survived. + = died (hrs.).

All of the cultures except one gave results the same as when first isolated. The culture *bd* originally toxic had become nontoxic, though tests with cultures incubated for a longer period showed a low toxicity, the death of mice occurring after a period of delay.

All cultures, including six more single-cell cultures isolated from the nontoxic culture *c*, were again tested on mice a month later, and in this case the results were consistent throughout with those obtained when the single-cell isolations were first made, the culture *bd* proving fatal to mice in seven hours.

The study is of interest in that it has been demonstrated that certain individuals in cultures originally toxic may apparently spontaneously lose their property of producing toxin. Just what factors are concerned in this phenomenon are problematical. A slight difference in the composition or reaction of the medium suggests itself as a possible explanation; but successive transplants of nontoxic cultures have always proved nontoxic, and it thus appears that once a culture loses its property of producing toxin it is not regained, regardless of any slight difference in the medium. The passage of the cultures through fly larvæ (*Lucilia sericata*) failed to change the toxic properties of the two types, all toxic cultures remaining toxic

and all the nontoxic cultures remaining nontoxic at the end of the experiment. The results suggest the possibility of the transformation of toxic cultures of other toxin-producing anaerobes, such as the organisms of tetanus and of botulism, Types A and B, into nontoxic types.

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### **MOSQUITO CONTROL AT TRINIDAD ASPHALT LAKE, BRITISH WEST INDIES.**

According to reports made by the American consul at Trinidad, British West Indies, one of the most serious difficulties in the production of asphalt at the famous Trinidad Lake, and also with petroleum developments in the immediate vicinity, has been the high incidence of malaria. Conditions at the lake are extremely favorable for mosquito breeding. The asphalt lake is about 20 feet below the level of the surrounding ground surface, and is gradually being lowered by the mining operations, affording a natural catchment area for rain water, accumulations of which are found between the mound-shaped masses of asphalt on the lake and in "pitch holes," "pitch cones," and grassy bottomed pools and drains adjacent to the lake.

It has been found difficult both to drain off the water and to fill up the water holes. Ordinary dug drains are soon refilled by the slowly moving currents of asphalt; and if holes are filled, the material is soon drawn down underneath the surface. Electrically driven pumps are used to pump off water that is drained into a sump at one edge of the lake. These drains connect with the pits from which the asphalt is taken, but within a few days they close in and become flush with the surface of the lake.

During the dry season, from February to May, the ordinary roadside drains in the country about the lake tend to dry up, but breeding places for mosquitoes are perpetuated in pitch holes and seepages, a large number of which are found within a mile of the lake. Sometimes a "pitch cone" will develop within a few hours, and, expanding under the pressure of gas, finally bursts, leaving a depression which holds water.

Owing to the heretofore ineffectual drainage, about the only check to mosquito propagation has been that resulting from the presence of several varieties of larvæ-eating fish which are found in large numbers in the water holes. For a number of years a large asphalt company operating at the lake has made serious attempt at mosquito control, adopting remedial measures that seemed practicable, including drains to carry off the rainfall, the spraying of pools with crude oil, keeping the brush and grass cut, and screening the bungalows of the employees. In 1919 the company placed a medical officer in